

I claim:

1. A variant single chain human tissue-type plasminogen activator protein having R275
5 and at least one other basic amino acid residue in the serine protease region substituted
by a non-basic amino acid residue thereby disrupting the salt bridge interaction
between aspartate 477 and lysine 429.
2. The protein of claim 1 wherein the non-basic amino acid residue is chosen from the
group consisting of glycine, serine, threonine, asparagine, tyrosine, glutamine, aspartic
10 acid, and glutamic acid and having a zymogenicity of at least 10.
3. The protein of claim 1 having a zymogenicity of at least 50.
4. The protein of claim 1 having a zymogenicity of at least 100.
5. The protein of claim 1 having a fibrin stimulation factor of at least 10,000.
6. The protein of claim 1 having a fibrin stimulation factor of at least 20,000.
- 15 7. The protein of claim 1 having a fibrin stimulation factor of at least 10,000.
8. The protein of claim 2 having a fibrin stimulation factor of at least 20,000.
9. The protein of claim 3 having a fibrin stimulation factor of at least 20,000.
10. The protein of claim 1 wherein the protein is at least a factor of 5 less inhibited by
PAI-1 compared to wild type single chain human tissue-type plasminogen activator
20 protein.
11. The protein of claim 1 wherein the protein is at least a factor of 9 less inhibited by
PAI-1 compared to wild type single chain human tissue-type plasminogen activator
protein.
12. The protein of claim 1 wherein the protein is at least a factor of 200 less inhibited by
25 PAI-1 compared to wild type single chain human tissue-type plasminogen activator
protein.
13. The protein of claim 8 wherein the protein is at least a factor of 9 less inhibited by
PAI-1 compared to wild type single chain human tissue-type plasminogen activator
protein.
- 30 14. The protein of claim 8 wherein the protein is at least a factor of 200 less inhibited by
PAI-1 compared to wild type single chain human tissue-type plasminogen activator
protein.
15. The protein of claim 1 wherein the protein has a fibrin selectivity factor of at least 100.
16. The protein of claim 8 wherein the protein has a fibrin selectivity factor of at least 100.

17. The protein of claim 14 wherein the protein has a fibrin selectivity factor of at least 100.
- 5 18. A polynucleotide encoding the protein of claim 1.
19. An expression vector comprising the polynucleotide of claim 18.
20. A cell comprising the expression vector of claim 19.
21. A variant single chain human tissue-type plasminogen activator protein selected from the group consisting of R275E,H417D, R275E,H417E and R275E,K429Y.
- 10 22. A polynucleotide encoding the protein of claim 21.
23. An expression vector comprising the polynucleotide of claim 22.
24. A cell comprising the expression vector of claim 23.
25. A composition for the treatment of an thrombotic condition comprising a physiologically effective amount of the protein of claim 1 in a pharmaceutically
15 suitable excipient.
26. The composition of claim 25 wherein the dose of the protein is from about 0.05 milligram per kilogram body weight to about 0.2 milligrams per kilogram body weight.
27. A diagnostic kit comprising antibodies to the protein of claim 1.
- 20 28. A diagnostic kit comprising the protein of claim 1.
29. A diagnostic kit comprising polynucleotides capable of hybridizing to the polynucleotide of claim 18.
30. A method of making a variant single chain human tissue-type plasminogen activator protein comprising the steps of culturing the cell of claim 24.
- 25 31. The method of claim 30 further comprising the additional step of purifying the protein.